FORM PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE (REV 10.95)

ATTORNEY'S DOCKET NUMBER
514485-3810

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO. (If known see 37 C.F.R. 1.5)
09/509051

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	(EARLIEST) PRIORITY DATE CLAIMED
PCT/EP98/06001	09/21/98	09/22/97

TITLE OF INVENTION: **ADDRESSABLE MODULAR RECOGNITION SYSTEM, PRODUCTION MODE AND USE**

APPLICANTS FOR DO/EO/US: **Christian MICULKA, Norbert WINDHAB AND Hans-Ulrich HOPPE**

Applicants herewith submit to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☐ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)), including 10 sheets of formal drawings and a copy of the International Search Report.
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventors (35 U.S.C. 371(c)(4)).
10. ☐ The annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:
    PCT/IB/306, PCT/IPEA/409, 416
    PCT/ISA/210

EXPRESS MAIL
Mailing Label Number: EL375197525US
Date of Deposit: March 20, 2000

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" Service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents and Trademarks, Box PCT Washington, DC 20231.

DAVID SKINNER
(Typed or printed name of person mailing paper or fee)

David Skinner
(Signature of person mailing paper or fee)

jmm20272021

U.S. APPLICATION NO.(If known, see 37 C.F.R. 1.50)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NO.
09/509051	PCT/EP98/06001	514485-3810

17. ☒ The following fees are submitted: (CALCULATIONS /PTO USE ONLY

Basic National Fee (37 CFR 1.492(a)(1)-(5):

Search Report has been prepared by the EPO or JPO.....$840.00 ($840.00

International preliminary examination fee paid to USPTO (37 CFR 1.482) $700.00

No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))...$770.00

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37CFR 1.445(a)(2) paid to USPTO.....$1040.00

International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4).....$96.00

ENTER APPROPRIATE BASIC FEE AMOUNT = ($ 840.00

Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)). ($ /

26 Claims	/Number Filed	/	Number Extra	/Rate	(
Total Claims	/ 32 - 20 =	/	12	/X $18.00	($ 216.00/
Independent Claims	/ 1 - 3 =	/	0	/X $78.00	($ /
Multiple dependent claim(s) (if applicable)				/+ $260.00	($ /

TOTAL OF ABOVE CALCULATIONS = ($1,056.00

Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28). (/ (/

SUBTOTAL = ($ 1,056.00

Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).+ (/ ($ /

TOTAL NATIONAL FEE = ($1,056.00

Fee for recording the enclosed assignments (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **$40.00** per property + ($ / ($ /

TOTAL FEES ENCLOSED = **($1,056.00**

(Amount to be: / (refunded /$

(charged /$

a. ☒ Our check in the amount of $1,056.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 50-0320 in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **50-0320**. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

WILLIAM F. LAWRENCE
FROMMER LAWRENCE & HAUG LLP
745 FIFTH AVENUE
NEW YORK, NEW YORK 10151

Dated: March 20, 2000

SIGNATURE

WILLIAM F. LAWRENCE
NAME

28,029
REGISTRATION NUMBER

Form PTO-1390 (REV **10-96**)

jmm20272021

514485-3810

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	:	MICULKA et al.
U.S. Serial No.	:	Filed Concurrently Herewith
Int'l Appln. No.	:	PCT/EP98/05998
Int'l Filing Date	:	21 SEPTEMBER 1998
Earliest Priority Date	:	22 SEPTEMBER 1997
For	:	METHOD FOR PRODUCING A PENTOPYRANOSYL NUCLEOSIDE

745 Fifth Avenue
New York, NY 10151

EXPRESS MAIL

Mailing Label Number: EL375197534US

Date of Deposit: March 20, 2000

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" Service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Box PCT, Washington, D.C. 20231.

DAVID SKINNER
(Typed or printed name of person mailing paper or fee)

David Skinner
(Signature of person mailing paper or fee)

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Box PCT
Washington, D.C. 20231

Dear Sir:

Preliminary to the examination of this U.S. national phase application, please enter the following amendments:

IN THE CLAIMS:

Claim 4, line 1, delete "one of Claims 1-3" and insert --Claim 1--;

Claim 7, line 1, delete "one of Claims 4-6" and insert --Claim 4--;

Claim 8, line 1, delete "one of Claims 4-7" and insert --Claim 4--;

Claim 9, line 1, delete "one of Claims 4-8" and insert --Claim 4--;

Claim 10, line 1, delete "one of Claims 4-9" and insert --Claim 4--;

Claim 11, line 1, delete "one of Claims 1-10" and insert --Claim 1--;

Claim 13, line 1, delete "or 12";

Claim 14, line 1, delete "one of Claims 11-13" and insert --Claim 11--;

Claim 16, line 1, delete "one of Claims 11-15" and insert --Claim 11--;

Claim 17, line 1, delete "one of Claims 1-16" and insert --Claim 1--;

Claim 19, line 1, delete "one of Claims 1-18" and insert --Claim 4--;

Claim 20, line 1, delete "one of Claims 1-19" and insert --Claim 4--;

Claim 21, line 1, delete "or 20";

Claim 23, line 1, delete "or 22";

Claim 24, line 1, delete "one of Claims 19-23" and insert --Claim 19--;

Claim 26, line 1, delete "one of Claims 1-25" and insert --Claim 1--;

Claim 27, line 2, delete "one of Claims 1-26" and insert --Claim 1--;

Claim 29, line 1, delete "or 2";

Claim 30, line 1, delete "one of Claims 27-29" and insert --Claim 27--; and

Claim 32, line 1, delete "one of Claims 1-26" and insert --Claim 1--.

REMARKS

This application includes multiple claim dependencies. The amendment removes the multiple claim dependencies, and the filing fee for this application was computed on the basis that no dependent claim depends from more than one preceding claim.

Entry of this amendment and an early examination on the merits are respectfully solicited.

Respectfully submitted,
FROMMER LAWRENCE & HAUG LLP

By: 

William F. Lawrence
Reg. No. 28,029
(212) 588-0800

Hoechst Aktiengesellschaft

H26102PCT BÖ/JK/sa

Addressable modular recognition system, its preparation and use

The present invention relates to a recognition system comprising

(a) at least one immobilized binding component A having at least one binding site for the recognition species B and

(b) at least one recognition species B which can bind to the binding component A and contains at least one binding site for a substrate S, the binding of the binding component A to the recognition species B taking place in the form of a molecular pairing system.

Arrays are arrangements of immobilized recognition species which play an important role in the simultaneous determination of analytes, especially in analytical methods and diagnosis. Examples are peptide arrays (Fodor et al., Nature 1993, 364, 555) and nucleic acid arrays (Southern et al. Genomics 1992, 13, 1008; U.S. Patent No. 5,632,957).

In experimental analytical systems, arrays permit particularly simple, rapid and reproducible data analysis as a result of the localized generation of events. Examples of this extend from the physical multi-channel detector as far as microtitre plates in laboratory medicine.

Arrays also serve for the storage and processing of information and are the fundamental construction element of nanotechnology.

Further important application areas can be found in biology, biochemistry, medicine and pharmacology. Thus, EP-A1-0 461 462 describes an immunoassay in which antigens which are positioned and immobilized in a field-like manner are brought into contact with one or more antibodies. WO 96/01836 describes, for example, an array of DNA molecules of differing sequence, which was used for the detection of gene sections and thus led, for example, to the diagnosis of pathogenic bacteria.

Immobilization by means of supramolecular interactions is also known outside of the array applications. Thus, excipients having anti-antibodies can be immobilized by means of an antigen bonded covalently to the excipient. The analytical system of immunoassays is based largely on enzyme immunoassays (EIAs), in which an enzymatically catalysed reaction indicates the presence of an antigen-antibody or an antigen-antibody-anti-antibody complex. One of the units involved in the complex is in this case either immobilized on a carrier or itself a carrier, e.g. in the form of tissue constituents.

Signal amplification processes of this type, however, have disadvantages, in particular with respect to the reliability of the qualitative information and quantification. A particular disadvantage of miniaturized arrays is the outlay and the costs in preparation.

The object of the present invention was therefore to find a recognition system which is simple, reliable, highly selective and moreover inexpensive.

The present invention therefore relates to a recognition system comprising
(a) at least one immobilized binding component A having at least one binding site for the recognition species B and
(b) at least one recognition species B which can bind to the binding component A and contains at least one binding site for a substrate S, the binding of the binding component A to the recognition species B taking place in the form of a molecular pairing system.

Such pairing systems are supramolecular systems of non-covalent interaction, which are distinguished by selectivity, stability and reversibility, and their properties are preferably influenced thermodynamically, i.e. by temperature, pH and concentration. Such pairing systems can also be used, for example, on account of their selective properties as "molecular adhesive" for the bringing together of different metal clusters to give cluster associates having potentially novel properties [see, for example, R. L. Letsinger, et al., Nature 1996, 382, 607-9; P. G. Schultz et al., Nature 1996, 382, 609-11].

It is therefore particularly advantageous if the pairing system is a complex which is formed by association of the binding component A with the recognition species B via non-covalent interactions. The non-covalent interactions are, in particular, hydrogen bridges, salt bridges, stacking, metal ligands, charge-transfer complexes and hydrophobic interactions.

In a particular embodiment, the molecular pairing system according to the present invention contains a nucleic acid and its analogues, in particular in the form of a pentose, preferably of a pentopyranose or pentofuranose. In general, the pentose is selected from a ribose, arabinose, lyxose or xylose. Pyranosyl-RNA (p-RNA), nucleic acid having one or more aminocyclohexylethanoic acid (CNA) units, peptide nucleic acid (PNA), or a nucleic acid having one or more [2-amino-4-(carboxymethyl)cyclohexyl]nucleobases is particularly preferred. Pyranosyl nucleic acids (p-NAs) and especially p-RNAs are particularly preferred.

p-NAs are in general structural types isomeric to the natural RNA, in which the pentose units are present in the pyranose form and are repetitively linked by phosphodiester groups between the positions C-2' and C-4'. "Nucleobase" is understood here as meaning the canonical nucleobases A, T, U, C, G, but also the pairs isoguanine/isocytosine and 2,6-diaminopurine/xanthine and within the meaning of the present invention also other purines and pyrimidines such as purine, 2,6-diaminopurine, 6-purinethiol, pyridine, pyrimidine, isoguanine, 6-thioguanine, xanthine, hypoxanthine, isocytosine, indole, tryptamine, N-phthaloyltryptamine, caffeine, theobromine, theophylline, benzotriazole or acridine, and preferably ribopyranosyladenosine, ribopyranosylguanosine, ribopyranosylthymidine, ribopyranosylcytosine, ribopyranosyltryptamine or ribopyranosyl-N-phthalotryptamine, ribopyranosyluracil or their [2-amino-4-(carboxymethyl)ribopyranosyl] derivatives.

p-NAs, namely the p-**R**NAs derived from **r**ibose, were described for the first time by Eschenmoser et al. (see Pitsch, S. et al. Helv. Chim. Acta 1993, 76, 2161; Pitsch, S. et al. Helv. Chim Acta 1995, 78, 1621; Angew. Chem. 1996, 108, 1619-1623). They form exclusively so-called Watson-Crick-paired, i.e. purine-pyrimidine- and purine-purine-paired, antiparallel, reversibly "melting", quasi-linear and stable duplices. Homochiral p-RNA strands of the opposite sense of chirality likewise pair controllably and are strictly non-helical in the duplex formed. This specificity, which is valuable for the construction of supramolecular units, is connected with the relatively low flexibility of the ribopyranose phosphate backbone and with the strong inclination of the base plane to the strand axis and the tendency resulting from this for intercatenary base stacking in the resulting duplex and can finally be attributed to the participation of a 2',4'-cis-disubstituted ribopyranose ring in the construction of the backbone.

These significantly better pairing properties make p-NAs preferred pairing systems for use in the construction of supramolecular units compared with DNA and RNA. They form a pairing system which is orthogonal to natural nucleic acids, i.e. they do not pair with DNAs and RNAs occurring in the natural form, which is advantageous, in particular, in the diagnostic field.

p-NAs are therefore particularly suitable for use in the field of nanotechnology, for example for the preparation of novel materials, diagnostics and therapeutics and also microelectronic, photonic or optoelectronic components and for the controlled bringing together of molecular species to give supramolecular units, such as, for example, for the (combinatorial) synthesis of protein assemblies [see, for example, A. Lombardi, J. W. Bryson, W. F. DeGrado, Biomoleküls (Pept. Sci.) 1997, 40, 495-504], as p-NAs, and particularly p-RNAs, form pairing systems which are strongly and thermodynamically controllable. A further application therefore results especially in the diagnostic and drug discovery field due to the possibility of providing functional, preferably biological, units such as proteins or DNA/RNA sections, e.g. with a p-RNA code which does not interfere with the natural nucleic acids (see, for example, WO93/20242).

According to the present invention, the length of the nucleic acid and its analogues is at least about 4-50, preferably at least about 4-25, in particular at least about 4-15, especially at least about 4-10, nucleotides.

In general, the binding component A is immobilized on a carrier.

The term "immobilized" is understood within the meaning of the present invention as meaning the formation of a covalent bond, quasi-covalent bond or supramolecular bond by association of two or more molecular species such as molecules having a linear constitution, in particular peptides, peptoids, proteins, linear oligo- or polysaccharides, nucleic acids and their analogues, or monomers such as heterocycles, in particular nitrogen heterocycles, or molecules having a non-linear constitution such as branched oligo- or polysaccharides or antibodies and their functional moieties. Functional moieties of antibodies are, for example, Fv fragments (Skerra & Plückthun (1988) Science 240, 1038), single-chain Fv fragments (scFv; Bird et al. (1988), Science 242, 423; Huston et al. (1988) Proc. Natl. Acad. Sci. U.S.A., 85, 5879) or Fab fragments (Better et al. (1988) Science 240, 1041).

The attachment to the carrier is thus in general carried out covalently, quasi-covalently, supramolecularly or physically, such as magnetically (A. R. Shepard et al. (1997) Nucleic Acids Res., 25, 3183-3185, No. 15), in an electrical field or through a molecular sieve. The binding component A is thereby either synthesized directly at the position of the carrier or "linked" to certain positions of the carrier. Examples are conjugation and carrier processes via periodote oxidation and reductive amination of the Schiff's base, N-hydroxysuccinimide esters of, preferably, dicarboxylic acid linkers, ethylenediaminephosphoamidate linkers, mercapto-, iodoacetyl or maleimido processes and/or covalent or non-covalent biotin linker processes.

The term "carrier" is understood within the meaning of the present invention as meaning a material, in particular chip material, which is present in solid or alternatively gelatinous form. Suitable carrier materials are, for example, ceramic, metal, in particular noble metal, glasses, plastics, crystalline materials or thin layers of the carrier, in particular of the materials mentioned, or (bio)molecular filaments such as cellulose, structural proteins.

A particular embodiment is therefore a recognition system according to the invention, in which the binding component A is immobilized on a carrier by means of a covalent bond, quasi-covalent bond or supramolecular bond by association of two or more molecular species such as molecules of linear constitution, in particular peptides, peptoids, proteins, linear oligo- or polysaccharides, nucleic acids and their analogues, or monomers such as heterocycles, in particular nitrogen heterocycles, or molecules of non-linear constitution such as branched oligo- or polysaccharides or antibodies and their functional moieties such as Fv fragments, single-chain Fv fragments (scFv) or Fab fragments.

In a further embodiment, the binding component A is immobilized at defined sites of the carrier, in particular in the form of a matrix, the defined sites of the carrier preferably being addressed.

According to the preferred recognition system, a molecule in the mobile (buffer) phase having the appropriate complementary sequence will only spontaneously form a supramolecular complex at the position of the suitable address. If further units having particular functions such as, for example, that of an antibody, are bonded to these mobile complementary addresses by chemical (conjugates) or supramolecular compound formation (complexes), depending on the address

pattern used a different functional array will be spread on the same immobilizate array.

The great advantages of such a modular system are the identical one-off provision of the carrier units for very different applications and the *in situ* generation of non-keepable bioconjugates, for example, from proteins, enzymes or living cells and the pairing radical.

A further advantage is the stepwise production of substrate binding event and the measurable binding event at the carrier position, i.e. the substrate can form a first complex with the soluble, addressed component (recognition species B) in a completely unhindered manner and then immobilize on the binding component A in a pairing manner in the space of the carrier position.

It is further particularly preferred if the binding component A is immobilized on a carrier electrode of the carrier, since an electronically readable signal is produced, for example, by a signal amplification of the impedance behaviour of carrier electrodes during binding events. Appropriate electrode processes are described in R. P. Andres (1996) Science, 272, 1323-1325 and appropriate impedance measurements are described in M. Stelzle et al. (1993) J. of Physical Chem., 97, 2974-2981.

A suitable recognition species B is, for example, a biomolecule which, for example, is selected from a peptide, peptoid, protein, such as receptor or functional moieties thereof such as the extracellular domain of a membrane receptor, antibodies or functional moieties thereof such as Fv fragments, single-chain Fv fragments (scFv) or Fab fragments, or cell constituents such as lipids, glycoproteins, filament constituents, or viruses, viral constituents such as capsids, or viroids, or their derivatives such as acetates and their active moieties, or substance libraries such as ensembles of structurally differing compounds, preferably oligomeric or polymeric peptides, peptoids, saccharides, nucleic acids.

The biomolecule customarily contains a binding region for the binding component A, which is preferably one of the nucleic acids described above or their analogues. In general, the biomolecule is bonded here to a selected nucleic acid or analogue via a linker. For example, a uracil-based linker is suitable, in which the 5-position of the uracil has preferably been modified, for example N-phthaloylaminoethyluracil, but also an indole-based linker, preferably tryptamine derivatives, such as, for example, N-phthaloyltryptamine.

In a particular embodiment, the immobilized binding component A contains various binding sites for various recognition species B, by means of which various recognition species B can bind to the binding component A.

In a further embodiment, at least one further recognition species B is immobilized on the binding component A.

Therefore a further recognition system according to the invention is characterized in that it comprises

(a) at least one immobilized binding component A having at least 2+n different binding sites for at least 2+n different recognition species B1, B2 ... Bn and a further recognition species B(n+3) different from the recognition species B1, B2 ... Bn, which is immobilized on the immobilized binding component A, and

(b) at least (n+3) different recognition species B1, B2 ... B(n+3),

where n is an integer from 0-20, preferably 0-10, in particular 0-5, especially 0 or 1.

In a further embodiment, the recognition species B1, B2 ... Bn originates from a substance library.

For the structural analysis of a complex of a substance library, it is particularly advantageous if the structure of the recognition species B(n+3) is known, and/or the different recognition species B recognize the same substrate S.

The term "substrate" is understood within the meaning of the present invention as meaning a non-carrier-bonded substance, which is intended to be recognized by the recognition system according to the invention. The substrate S is in general selected from molecules, preferably pharmaceuticals and plant protection active compounds, metabolites, physiological messenger substances, derivatives of lead structures, substances which are produced or produced to an increased extent in the human or animal body in the case of pathological changes, or transition state analogues, or peptides, peptoids, proteins such as receptors or functional moieties thereof such as the extracellular domain of a membrane receptor, antibodies or functional moieties thereof such as Fv fragments, single-chain Fv fragments (scFv) or Fab fragments, or cell constituents such as lipids, glycoproteins, filament constituents, or viruses, viral constituents such as capsids, or viroids, or their derivatives such as acetates, or monomers such as heterocycles, in particular nitrogen heterocycles, or molecules of non-linear constitution such as branched

oligo- or polysaccharides, or substance libraries such as ensembles of structurally differing compounds, preferably oligomeric or polymeric peptides, peptoids, saccharides, nucleic acids, esters, acetals or monomers such as heterocycles, lipids, steroids, or targets for pharmaceuticals, preferably pharmaceutical receptors, voltage-dependent ion channels, transporters, enzymes or biosynthesis units of microorganisms.

Substance libraries are known to the person skilled in the art from the field of combinatorial chemistry. Examples are the readily accessible peptide libraries, produced by permutation of the peptide sequence. If such libraries pair, completely novel supramolecules or complexes result. The appreciable number of possible complexes possibly includes recognition regions for substrate molecules, similarly to the epitope of an antibody. The embodiment then permits screening of such a stochastic binding event. If one of the conjugate libraries is bonded to the carrier, its identity (e.g. the peptide sequence) can be directly fixed by the codon address or, if the address is constant, by its mere position. The array produces a so-called encoded library for one of the pairing strands and simplifies the complex analysis of the supramolecular library.

In a further preferred embodiment, the recognition system according to the invention is an immunoassay.

Another subject of the present invention is also a process for the identification of a substrate S in a sample with the aid of the recognition system according to the invention, in which

(a) a recognition species B which recognizes the substrate S is brought into contact with the sample,

(b) is simultaneously or successive brought into contact with an immobilized recognition species B, and

(c) the formation of a complex of immobilized binding component A, recognition species B and substrate S is detected.

In particular, in the process according to the invention the formation of the complex is controlled by means of physical parameters such as temperature, salts, solvents, electrophoretic processes.

In general, the complex formed is detected by means of labelling such as radioactive or fluorescent labelling, enzymatic labelling, redox labelling, spin labelling of the recognition species B, or by means of the complex itself, for

example by means of electrode processes such as by means of chemical processes, e.g. redox processes in the environment or on the electrode or by means of a physical parameter such as by means of impedance measurement or direct current measurement.

Particular amplification or preconcentration steps of the substrates are thus not needed for many applications, which is particularly advantageous. The chemical and physical heterogeneity of the positions before and after the pairing events can moreover be eliminated using the direct electronic process, very advantageously by parametrization or calibration by means of the software.

The problem that important substrate molecules for such applications can be molecules of the natural pairing systems DNA and RNA themselves and would thus interact interferingly with the addressing is solved in that particularly stable, selective and non-natural pairing systems, such as, for example, p-NAs, are used.

The present invention therefore also relates to a process with which recognition species, preferably natural DNA or RNA strands and proteins, in this case preferably antibodies or functional moieties of antibodies, are clearly encoded by p-NA sections, preferably p-RNA sections. These can then be hybridized with the associated codons on a solid carrier. Thus always novel, diagnostically useful arrays can be constructed on a solid carrier, which is equipped with codons in the form of an array only by adjustment of hybridization conditions with always novel combinations of recognition species at the desired positions. If the analyte, for example a biological sample such as serum or the like, is then applied, the species to be detected are then bonded to the array in a certain pattern, which is then recorded indirectly (e.g. by fluorescence labelling of the recognition species) or directly (e.g. by impedance measurement at the linkage point of the codons). The hybridization is then eliminated by means of suitable conditions (temperature, salts, solvent, electrophoretic processes), so that again only the carrier with the codons remains. This is then again loaded with other recognition species and is used, for example, for the same analyte for the determination of another sample. The always novel arrangement of recognition species in the array format and the use of p-NAs as pairing systems is particularly advantageous compared with other systems, see, for example, WO 96/13522.

In the process according to the invention, the complex of recognition species B and substrate S can also be isolated in a further step. For this, for example, the complex

is isolated from recognition species B and substrate S after freezing the binding equilibrium or covalent cross-linking of recognition species B and substrate S.

The recognition system according to the invention is consequently particularly highly suitable for finding a substrate S for diagnosis, for the preparation of a catalyst and/or for the preparation of an electronic component, in particular for the finding, for the optimization and/or for the preparation of a pharmaceutical active compound or plant protection active compound.

Depending on the addresses synthesized, kits which form the test system by pairing on the existing codon array *in situ* can thus be rapidly assembled for different questions or diagnostic problems. Biomolecules, for example very generally cell or viral constituents, very particularly monoclonal antibodies or their functional moieties, are preferred.

The following figures are intended to describe the invention in greater detail, without restricting it.

DESCRIPTION OF THE FIGURES

Fig. 1 shows schematically the general principle of a recognition species, which is produced *in situ* around a substrate to be recognized. The complexing unit (peptide) can be known by a carrier matrix. A binding pocket formed under thermodynamic or kinetic control is formed here as a complex with the substrate. The pairing unit A complementary to all B units is immobilized on the carrier.

Fig. 2 shows schematically an arrangement of immobilized recognition structures (arrays) on a solid carrier.

Fig. 3 shows schematically the modular production of a supramolecular array. Different immunoarrays are constructed on the same anticodon carrier by addressing with the selective pairing regions.

Fig. 4 shows schematically the construction of an array having 4 carrier positions (electrodes) and the measuring principle.

Fig. 5 shows schematically the detection of the pairing of the anticodon-codon molecules by UV spectroscopy and impedance spectroscopy. By

lowering the temperature, the strands pair, the buffer supernatant weakens, the UV extinction of the supernatant decreases and the change in the electrode bilayer acts on the impedance measurement.

Fig. 6 shows schematically the functioning of an addressed immunoarray. Only electrode 3 carries the appropriate address for an antibody-pairing strand conjugate. If the appropriate antigen is added, the impedance at the electrode 1 changes other than by mere change of buffer at the other electrodes.

Fig. 7 shows the cooling curves of a temperature-induced UV pairing experiment with two complementary p-RNA addresses, to which a histidine peptide is conjugated in each case. The pairing produces a recognition region for nickel ions as a substrate. The substrate leads to a clear increase in the transition temperature T_m, which is not observed without the histidine radicals.

Fig. 8 shows schematically a simple matrix of two vapour-deposited gold electrodes.

Fig. 9 shows the direct electronic detection of an antigen-antibody complex at one electrode position of the array by impedance spectroscopy.

Fig. 10 shows an additional detection of the antigen-antibody complex at the addressed electrode by means of fluorescence.

Examples

Example 1

Synthesis of a p-RNA oligonucleotide containing a linker using linker of the formula 4′ AGGCAIndT 2′:

1.1 Solid-phase synthesis of the oligonucleotide

A, G, C, T are the nucleobases adenine, guanine, cytosine and thymine and Ind is aminoethylindole (indole CH_2-CH_2-NH_2) as a linker in the form of a nucleobase.

The fully automatic solid-phase synthesis was carried out with 15 µmol in each case. A synthesis cycle consists of the following steps:

(a) detritylation: 5 minutes with 6% DCA (dichloroacetic acid) in CH_2Cl_2 (79 ml).

(b) washing with CH_2Cl_2 (20 ml), acetonitrile (20 ml) and then flushing with argon:

(c) coupling: washing of the resin with the activator (0.5 M pyridine.HCl in CH_2Cl_2 (0.2 ml) and then 30 minutes' treatment with activator (0.76 ml) and phosphoramidite of the corresponding nucleobase (0.76 ml: 8 eq; 0.1 M in acetonitrile) in the ratio 1/1;

(d) capping: 2 minutes' treatment with 50% Cap A (10.5 ml) and 50% Cap B (10.5 ml) from PerSeptive Biosystems, Inc., Texas, USA (Cap A: THF, lutidine, acetic anhydride; Cap B: 1-methylimidazole, THF, pyridine);

(e) oxidation: 1 minute's treatment with 120 ml of iodine solution (400 mg of iodine in 100 ml of acetonitrile, 46 ml of H_2O and 9.2 ml of sym-collidine); and

(f) washing with acetonitrile (22 ml).

To facilitate the subsequent HPLC purification of the oligonucleotides, the last DMT (dimethoxytrityl) group was not removed. To detect the last coupling with the modified phosphoamidites, after the synthesis with 1% of the resin a trityl cation absorption was carried out in UV (503 nm).

1.2 Work-up of the oligonucleotide:

The removal of the allyl ether protective groups was carried out with a solution of tetrakis(triphenylphosphine)palladium (272mg), triphenylphosphine (272 mg) and diethylammonium hydrogencarbonate in CH_2Cl_2 (15ml) after 5 hours at RT. The glass carriers were then washed with CH_2Cl_2 (30ml), acetone (30ml) and water (30ml). In order to remove palladium complex residues, the resin was rinsed with an aqueous 0.1 M sodiumdiethyldithiocarbamate hydrate solution. The abovementioned washing operation was carried out once more in the reverse order. The resin was then dried in a high vacuum for 10 minutes. The removal step from the glass carrier with simultaneous debenzoylation was carried out in 24% hydrazine hydrate solution (6ml) at 4°C. After HPLC checking on RP 18 (18-25 hours), the oligonucleotide "Trityl ON" was freed of hydrazine by means of an activated (acetonitrile, 20 ml) Waters Sep-Pak cartridge. The hydrazine was washed with TEAB, 0.1M (30ml). The oligonucleotide was then eluted with acetonitrile/TEAB, 0.1M (10ml). It was then purified by means of HPLC for the removal of fragment sequences and the DMT deprotection (30 ml of 80% strength

aqueous formic acid) was carried out. Final desalting (by means of Sep-Pak cartridge, with TEAB buffer 0.1M/acetonitrile: 1/1) yielded the pure oligonucleotide.

Example 2

Iodoacetylation of *p*-RNA with N-(iodoacetyloxy)succinimide

p-RNA sequence: 4′ AGGCAIndT 2′ M_w = 2266.56 g/mol, prepared according to Example 1.

1 eq. of the *p*-RNA was dissolved (1 ml per 350 nmol) in a 0.1 molar sodium hydrogencarbonate solution (pH 8.4) and treated (40 µl per mg) with a solution of N-(iodoacetyloxy)succinimide in DMSO. The batch is blacked out with aluminium foil and allowed to stand at room temperature for 30-90 minutes.

The progress of the reaction was monitored by means of analytical HPLC. The standard conditions were:
Buffer A : 0.1 molar triethylammonium acetate buffer in water
Buffer B : 0.1 molar triethylammonium acetate buffer in water:acetonitrile 1:4
Gradient : starting from 10% B to 50% B in 40 minutes
Column material: 10 µM LiChrosphere ® 100 RP-18 from Merck Darmstadt GmbH; 250 x 4 mm
Retention time of the starting materials: 18.4 minutes
Retention time of the products in this case: 23.1 minutes

After reaction was complete, the batch was diluted to four times the volume with water. A Waters Sep-Pak cartridge RP-18 (from 15 oD 2 g packing) was activated with 2 x 10 ml of acetonitrile and 2 x 10 ml of water, the oligonucleotide was applied and allowed to sink in, and the reaction vessel was washed with 2 x 10 ml of water, rewashed with 3 x 10 ml of water in order to remove salt and reagent, and eluted first with 5 x 1 ml of 50:1 water:acetonitrile and then with 1:1. The product eluted in the 1:1 fractions in very good purity. The fractions were concentrated in the cold and in the dark, combined and concentrated again.

The yields were determined by means of UV absorption spectrometry at 260 nm.
Mass spectrometry:
Sequence : 4′ AGGCAInd($CH_2CH_2NHCOCH_2$-I)T 2′
calculated mass : 2434.50 g/mol

found mass MH_2^{2+}: 1217.9 g/mol = 2433

Example 3

Conjugation of *p*-RNA to a peptide of the sequence $(His)_6$:

The iodoacetylated *p*-RNA (M_w = 2434.50 g/mol) was dissolved in a buffer system (1000µl per 114 nmol) and then treated with a solution of the peptide in buffer (2 mol eq. of $(His)_6$ peptide).

Buffer system : Borax/HCl buffer from Riedel-de Haën, pH 8.0, was mixed in the ratio 1:1 with a 10 millimolar solution of EDTA disodium salt in water and adjusted to pH 6.3 with HCl. A solution which contained 5 mM Na_2EDTA was obtained thereby.

The batch was left at room temperature in the dark until reaction was complete. The reaction was monitored by means of HPLC analysis. After reaction was complete, the batch was purified directly by means of RP-HPLC. The fractions were concentrated in the cold and in the dark, combined and concentrated again. The residue was taken up in water and desalted. A Waters Sep-Pak cartridge of RP-18 (from 15 oD 2 g packing) was activated with 2 x 10 ml of acetonitrile and 2 x 10 ml of water, the oligonucleotide was applied and allowed to sink in, and the reaction vessel was washed with 2 x 10 ml of water, rewashed with 3 x 10 ml of water in order to remove the salt, and eluted with water:acetonitrile 1:1. The product fractions were concentrated, combined and concentrated again.

The yields were determined by means of UV absorption spectrometry at 260 nm. They reached 70-95% of theory.

HPLC Analysis:
Buffer A : 0.1 molar triethylammonium acetate buffer in water
Buffer B : 0.1 molar triethylammonium acetate buffer in water: acetonitrile 1:4
Gradient : starting from 10% B to 50% B in 40 minutes
Column material : 10 µM LiChrosphere ® 100 RP-18 from Merck Darmstadt GmbH; 250 x 4
Retention time of the product: 16.9 minutes

Mass spectrometry:

Sequence : 4′ AGGCAInd($CH_2CH_2NHCOCH_2$-$(His)_6$T 2′
calculated mass: MH_2^{2+} : 1626.9 g/mol
found mass MH_2^{2+} : 1626.0 g/mol

The complementary sequence 4′ Ind($CH_2CH_2NHCOCH_2$-$(His)_6$TGCCT 2′ was prepared analogously:

calculated mass MH_2^{2*}: 1436.2 g/mol
found mass MH_2^{2*} : 1436.4 g/mol

Peptide libraries for the formation of recognition regions on the p-RNA were also conjugated analogously.

It was possible to demonstrate in the UV solution experiment that the interaction of the histidine subunits with a substrate (nickel ions), by itself influences the pairing properties. A conjugate solution of in each case 5 µM p-RNA, in 10mM Tris HCl 150mM ultrapure NaCl showed a T_m of 32°C in the UV pairing experiment, which increased by 10° C to 42° after addition of 10 equivalents of nickel ions per strand. Thus the detection, i.e. the recognition of a substrate here very advantageously accompanies the addressing itself; this corresponds on the carrier matrix to the immobilization process.

Example 4

Direct electronic detection of an antibody/antigen recognition on the addressable recognition system.

A simple matrix of two vapour-deposited gold electrodes was used as an example of an addressable recognition system (see Fig. 8).

A commercially obtainable thiol-reduced antibody unit (Rockland Immuno-chemicals, Pennsylvania, USA) was conjugated to an iodoacetylated p-RNA sequence as described above.

The complementary p-RNA-unit 4'Ind--TAGGCAAT 2′ was thiol-activated on the amino linker by means of 100 equivalents of Traut's reagent in 1mM aqueous EDTA and borax buffer pH 9.5, purified by reverse-phase HPL chromatography after 6 hours, and bonded overnight to one of the two gold electrodes which had been freshly cleaned by means of UV light.. Only this electrode binds the antibody-p-RNA conjugate by pairing (see Fig. 9).

The figure shows the impedance signal (without further wiring; spectrometer Solarton Instruments 1260 interface; Solarton SI 1287) of the thio-reduced antibody, which was bonded directly overnight to a freshly cleaned electrode of the type described, before and after an antibody-antigen complexation of the immobilized antibody under the buffer conditions 1/15 mol/l Na_2HPO_4, KH_2PO_4, pH 7.4 and room temperature.

It was possible to check the recognition result in the selected case by means of fluorescent labels, as the commercially obtainable antigen (a human IgG-F(ab')2 fraction of Rockland Immunochemicals) is fluorescein-labelled (see Fig. 10).

Patent Claims

1. Recognition system comprising
(a) at least one immobilized binding component A having at least one binding site for the recognition species B and
(b) at least one recognition species B which can bind to the binding component A and contains at least one binding site for a substrate S, characterized in that the binding of the binding component A to the recognition species B takes place in the form of a molecular pairing system.

2. Recognition system according to Claim 1, characterized in that the pairing system is a complex which is formed by association of the binding component A with the recognition species B via non-covalent interactions.

3. Recognition system according to Claim 2, characterized in that the non-covalent interactions are selected from hydrogen bridges, salt bridges, stacking, metal ligands, charge-transfer complexes and hydrophobic interactions.

4. Recognition system according to one of Claims 1-3, characterized in that the molecular pairing system contains a nucleic acid and its analogues.

5. Recognition system according to Claim 4, characterized in that the nucleic acids and their analogues is a pentose, preferably a pentopyranose or pentofuranose.

6. Recognition system according to Claim 5, characterized in that the pentose is selected from a ribose, arabinose, lyxose or xylose.

7. Recognition system according to one of Claims 4-6, characterized in that the nucleic acid and its analogues is selected from pyranosyl-RNA (p-RNA), nucleic acid having one or more aminocyclohexylethanoic acid (CNA) units, peptide nucleic acid (PNA), or a nucleic acid having one or more [2-amino-4-(carboxymethyl)cyclohexyl]nucleobases.

8. Recognition system according to one of Claims 4-7, characterized in that the nucleobase of the nucleic acid or its analogues is selected from purine, 2,6-diaminopurine, 6-purinethiol, pyridine, pyrimidine, adenine, guanine, isoguanine, 6-thioguanine, xanthine, hypoxanthine, thymidine, cytosine, isocytosine, indole, tryptamine, N-phthaloyltryptamine, uracil, caffeine, theobromine, theophylline, benzotriazole or acridine.

9. Recognition system according to one of Claims 4-8, characterized in that the nucleic acid analogues are selected from ribopyranosyladenosine, ribopyranosylguanosine, ribopyranosylthymidine, ribopyranosylcytosine, ribopyranosyltryptamine or ribopyranosyl-N-phthalotryptamine, ribopyranosyl-uracil or their 2-amino-4-(carboxymethyl)ribopyranosyl] derivatives.

10. Recognition system according to one of Claims 4-9, characterized in that the length of the nucleic acid and its analogues is at least about 4-50, preferably at least about 4-25, in particular at least about 4-15, especially at least about 4-10, nucleotides.

11. Recognition system according to one of Claims 1-10, characterized in that the binding component A is immobilized on a carrier.

12. Recognition system according to Claim 11, characterized in that the carrier is selected from ceramic, metal, in particular noble metal, glasses, plastics, crystalline materials or thin layers of the carrier, in particular of the materials mentioned, or (bio)molecular filaments, such as cellulose, structural proteins.

13. Recognition system according to Claim 11 or 12, characterized in that the binding component A is immobilized on a carrier by means of a covalent bond, quasi-covalent bond or supramolecular bond by association of two or more molecular species such as molecules of linear constitution, in particular peptides, peptoids, proteins, linear oligo- or polysaccharides, nucleic acids and their analogues, or monomers such as heterocycles, in particular nitrogen heterocycles, or molecules of non-linear constitution such as branched oligo- or polysaccharides or antibodies and their functional moieties such as Fv fragments, single-chain Fv fragments (scFv) or Fab fragments.

14. Recognition system according to one of Claims 11-13, characterized in that the binding component A is immobilized at defined sites of the carrier, preferably in the form of a matrix.

15. Recognition system according to Claim 14, characterized in that the defined sites of the carrier are addressed.

16. Recognition system according to one of Claims 11-15, characterized in that the binding component A is immobilized on a carrier electrode of the carrier.

17. Recognition system according to one of Claims 1-16, characterized in that the recognition species B is a biomolecule.

18. Recognition system according to Claim 17, characterized in that the biomolecule is selected from peptide, peptoid, protein such as receptor or functional moieties thereof such as the extracellular domain of a membrane receptor, antibodies or functional moieties thereof such as Fv fragments, single-chain Fv fragments (scFv) or Fab fragments, or cell constituents such as lipids, glycoproteins, filament constituents, or viruses, viral constituents such as capsids, or viroids, or their derivatives such as acetates and their active moieties, or substance libraries such as ensembles of structurally differing compounds, preferably oligomeric or polymeric peptides, peptoids, saccharides, nucleic acids.

19. Recognition system according to one of Claims 1-18, characterized in that the immobilized binding component A contains various binding sites for various recognition species B, by means of which various recognition species B can bind to the binding component A.

20. Recognition system according to one of Claims 1-19, characterized in that at least one further recognition species B is immobilized on the binding component A.

21. Recognition system according to Claim 19 or 20, characterized in that it comprises

(a) at least one immobilized binding component A having at least 2+n different binding sites for at least 2+n different recognition species B1, B2 ... Bn and a further recognition species B(n+3) different from the recognition

species B1, B2 ... Bn, which is immobilized on the immobilized binding component A, and
(b) at least (n+3) different recognition species B1, B2 ... B(n+3),
where n is an integer from 0-20, preferably 0-10, in particular 0-5, especially 0 or 1.

22. Recognition system according to Claim 21, characterized in that the recognition species B1, B2 ... Bn originates from a substance library.

23. Recognition system according to Claim 21 or 22, characterized in that the structure of the recognition species B(n+3) is known.

24. Recognition system according to one of Claims 19-23, characterized in that the different recognition species B recognize the same substrate S.

25. Recognition system according to Claim 24, characterized in that the substrate S is selected from molecules, preferably pharmaceuticals and plant protection active compounds, metabolites, physiological messenger substances, derivatives of lead structures, substances which are produced or produced to an increased extent in the human or animal body in the case of pathological changes, or transition state analogues, or peptides, peptoids, proteins such as receptors or functional moieties thereof such as the extracellular domain of a membrane receptor, antibodies or functional moieties thereof such as Fv fragments, single-chain Fv fragments (scFv) or Fab fragments, or cell constituents such as lipids, glycoproteins, filament constituents, or viruses, viral constituents such as capsids, or viroids, or their derivatives such as acetates, or monomers such as heterocycles, in particular nitrogen heterocycles, or molecules of non-linear constitution such as branched oligo- or polysaccharides, or substance libraries such as ensembles of structurally differing compounds, preferably oligomeric or polymeric peptides, peptoids, saccharides, nucleic acids, esters, acetals or monomers such as heterocycles, lipids, steroids, or target structures for pharmaceuticals, preferably pharmaceutical receptors, voltage-dependent ion channels, transporters, enzymes or biosynthesis units of microorganisms.

26. Recognition system according to one of Claims 1-25, characterized in that it is an immunoassay.

27. Process for the identification of a substrate S in a sample with the aid of the recognition system according to one of Claims 1-26, characterized in that
(a) a recognition species B which recognizes the substrate S is brought into contact with the sample,
(b) is simultaneously or successively brought into contact with an immobilized recognition species B, and
(c) the formation of a complex of immobilized binding component A, recognition species B and substrate S is detected.

28. Process according to Claim 27, characterized in that the formation of the complex is controlled by means of physical parameters such as temperature, salts, solvents, electrophoretic processes.

29. Process according to Claim 27 or 28, characterized in that the complex is detected by means of labelling such as radioactive or fluorescent labelling, enzymatic labelling, redox labelling, spin labelling of the recognition species B, or by means of the complex itself, for example by means of electrode processes such as by means of chemical processes, e.g. redox processes in the environment or on the electrode or by means of a physical parameter such as by means of impedance measurement or direct current measurement.

30. Process according to one of Claims 27-29, characterized in that the complex of recognition species B and substrate S is isolated in a further step.

31. Process according to Claim 30, characterized in that the complex of recognition species B and substrate S is isolated after freezing the binding equilibrium or covalent cross-linking of recognition species B and substrate S.

32. Use of the recognition system according to one of Claims 1-26 for finding a substrate S for diagnosis, for the preparation of a catalyst and/or for the preparation of an electronic component, in particular for the finding, for the optimization and/or for the preparation of a pharmaceutical active compound or plant protection active compound.

Hoechst Aktiengesellschaft

H26102PCT BÖ/JK/sa

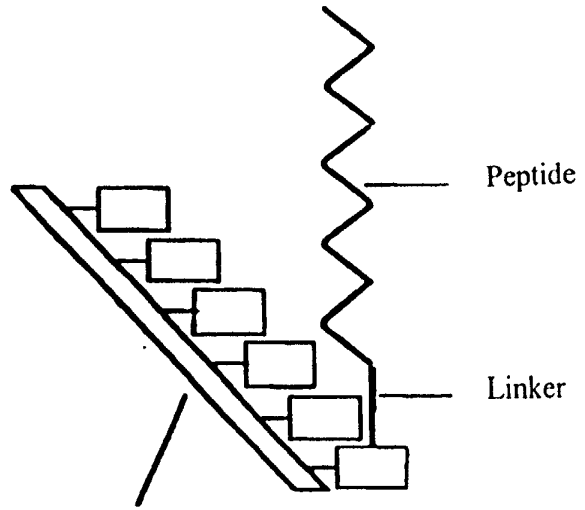
Abstract

The present invention relates to a recognition system comprising

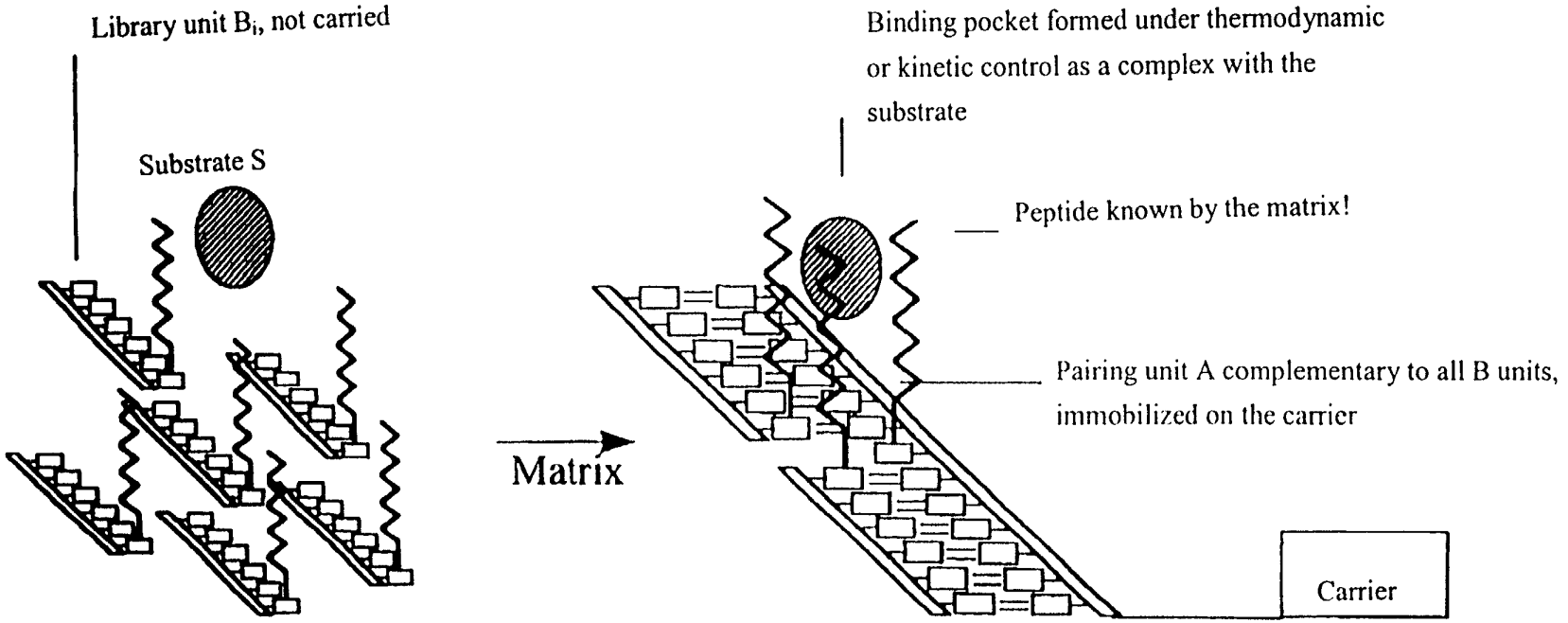
(a) at least one immobilized binding component A having at least one binding site for the recognition species B and

(b) at least one recognition species B which can bind to the binding component A and contains at least one binding site for a substrate S, the binding of the binding component A to the recognition species B taking place in the form of a molecular pairing system.

Fig. 1



Oligomer from pairing system



09/509051

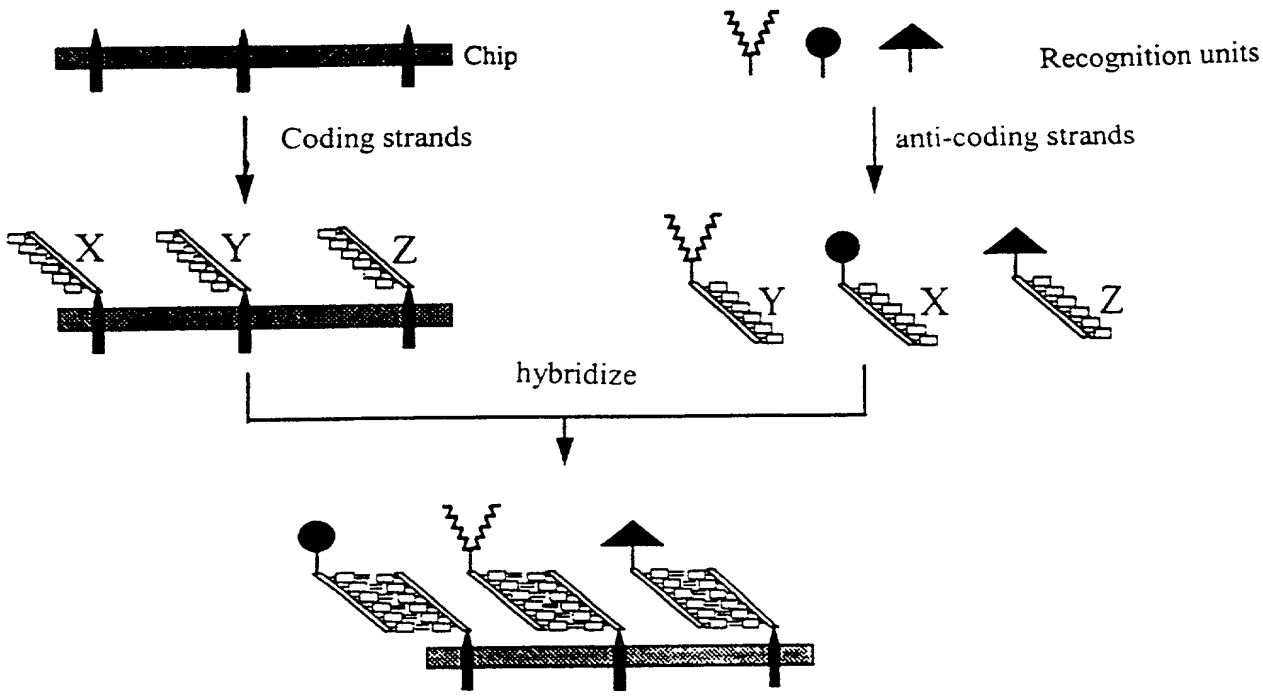

Chip
Recognition units
Coding strands
anti-coding strands
X
Y
Z
Y
X
Z
hybridize

Fig 2

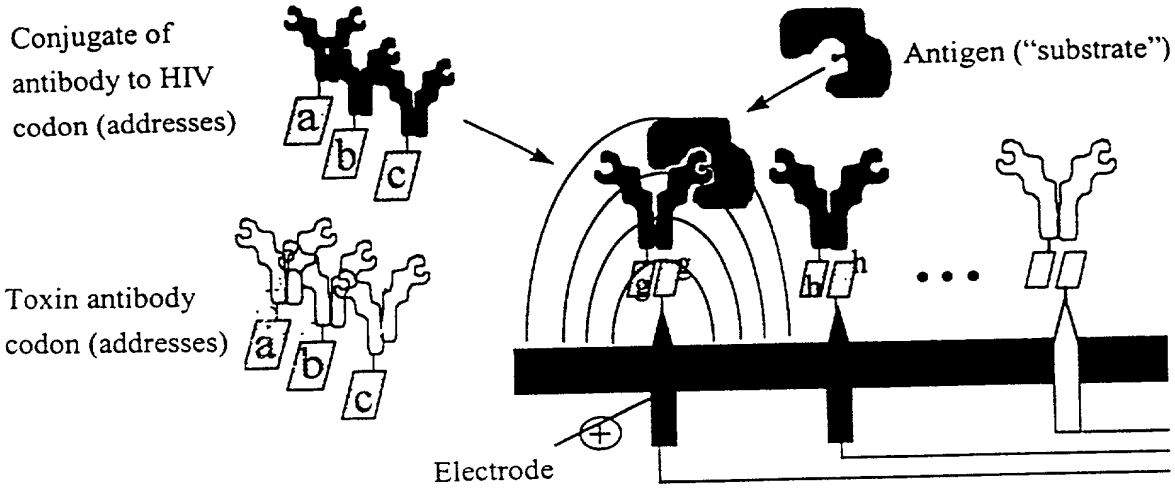
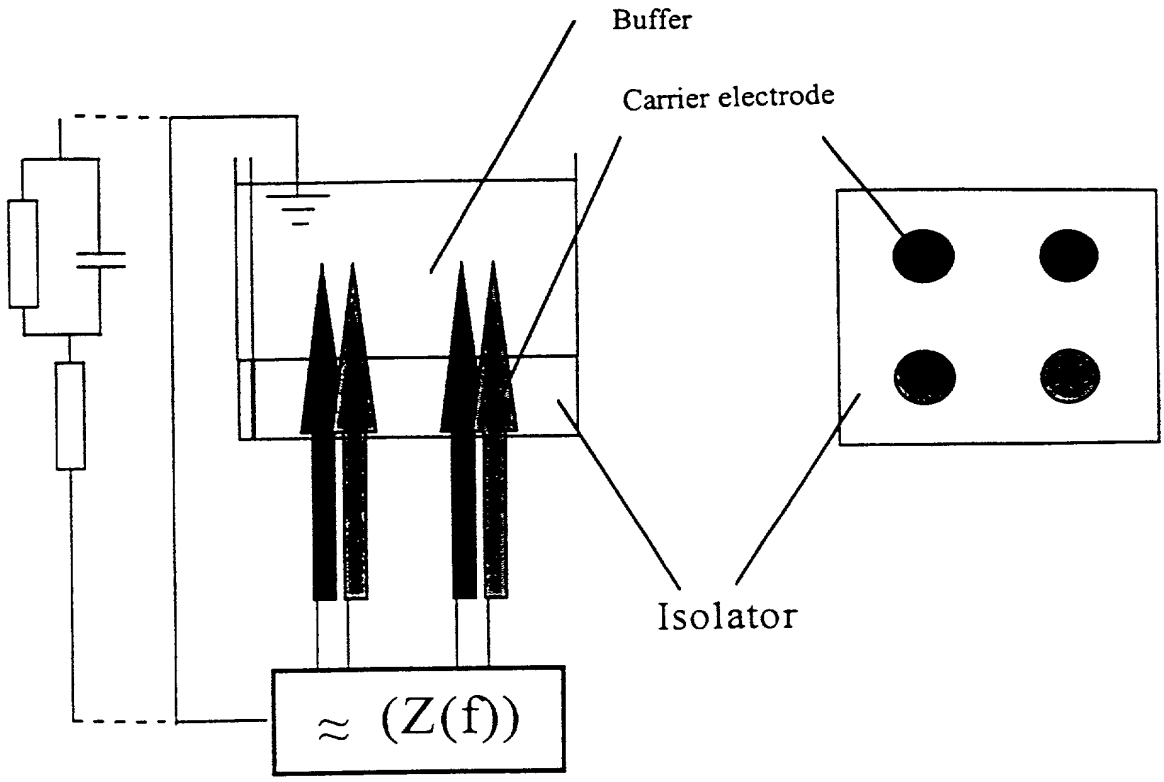

Conjugate of
antibody to HIV
codon (addresses)
a
b
c
Toxin antibody
codon (addresses)
a
b
c
Antigen ("substrate")
g
h
Electrode

Fig. 3



Idealized substitute circuit diagram of the experimental arrangement

Fig. 4

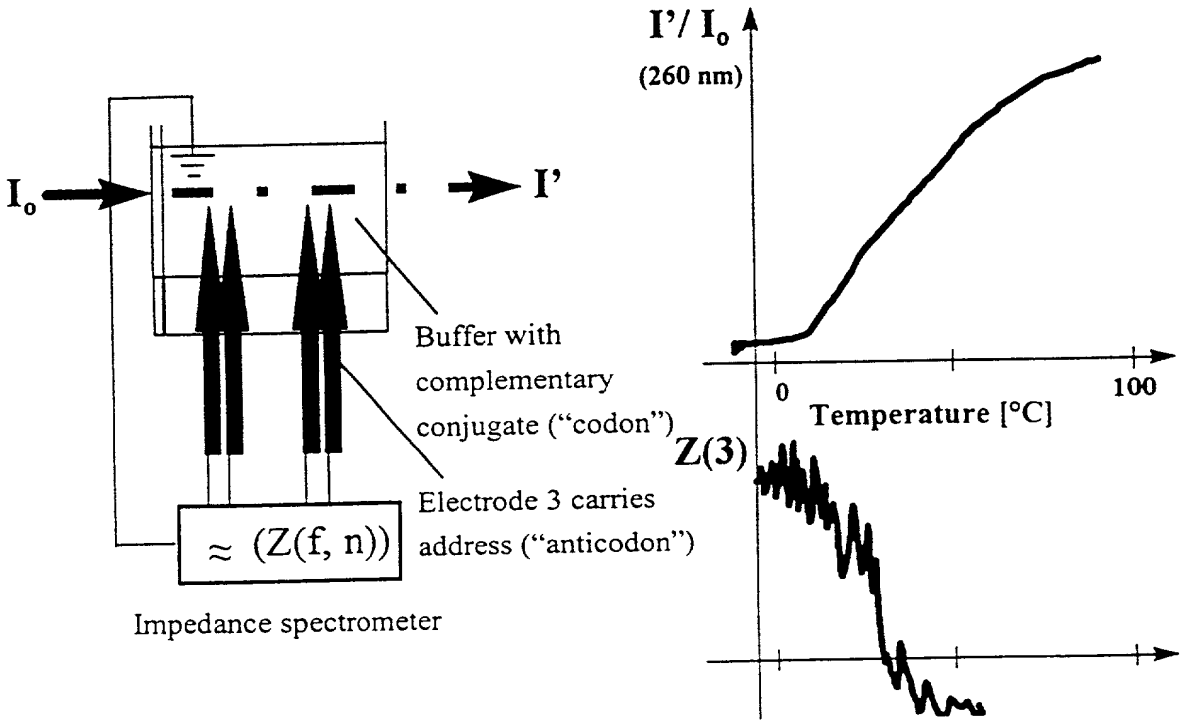

I'/ I0
(260 nm)
I0
I'
Buffer with
complementary
conjugate ("codon")
Electrode 3 carries
address ("anticodon")
≈ (Z(f, n))
Impedance spectrometer
0
100
Temperature [°C]
Z(3)

Fig. 5

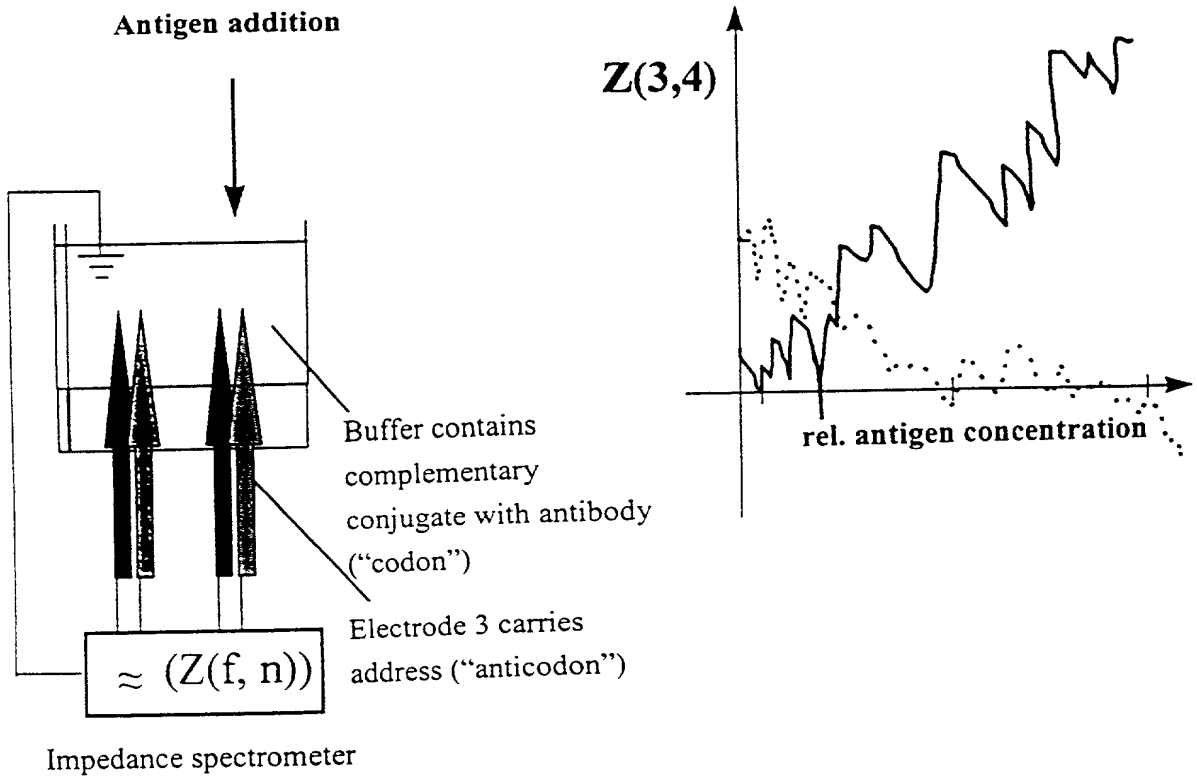

Antigen addition
Buffer contains complementary conjugate with antibody ("codon")
Electrode 3 carries address ("anticodon")
≈ (Z(f, n))
Impedance spectrometer
Z(3,4)
rel. antigen concentration

Fig. 6

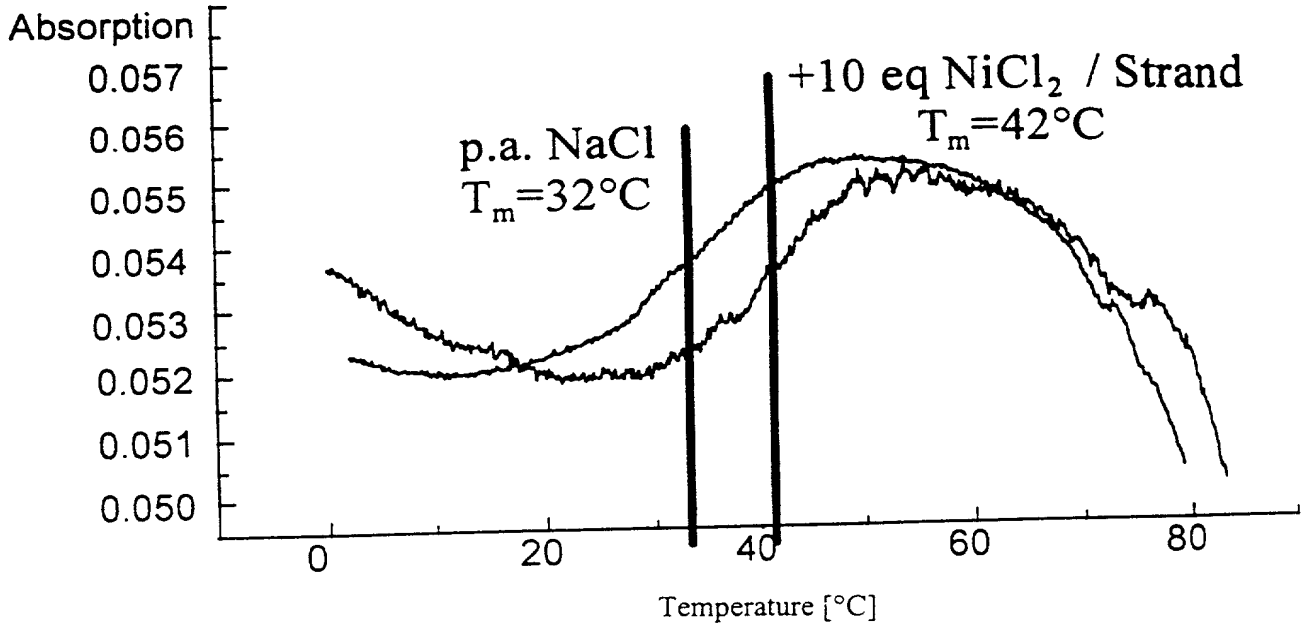

Absorption
0.057
0.056
0.055
0.054
0.053
0.052
0.051
0.050
0
20
40
60
80
Temperature [°C]
p.a. NaCl
T_m=32°C
+10 eq $NiCl_2$ / Strand
T_m=42°C

Fig. 7

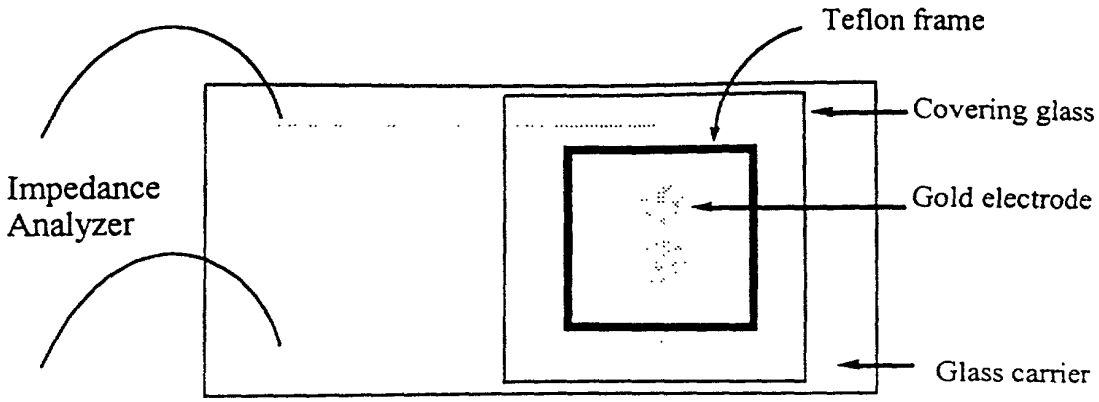


Fig. 8

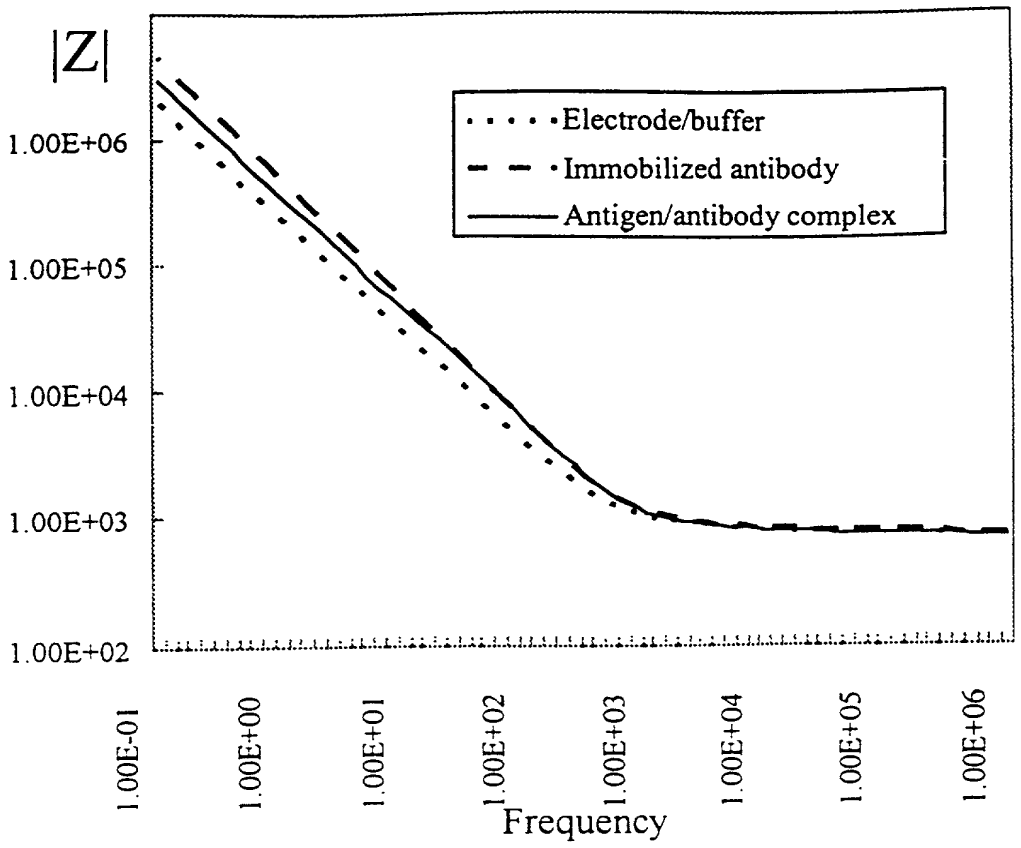

|Z|
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
Electrode/buffer
Immobilized antibody
Antigen/antibody complex
1.00E-01
1.00E+00
1.00E+01
1.00E+02
1.00E+03
1.00E+04
1.00E+05
1.00E+06
Frequency

Fig. 9

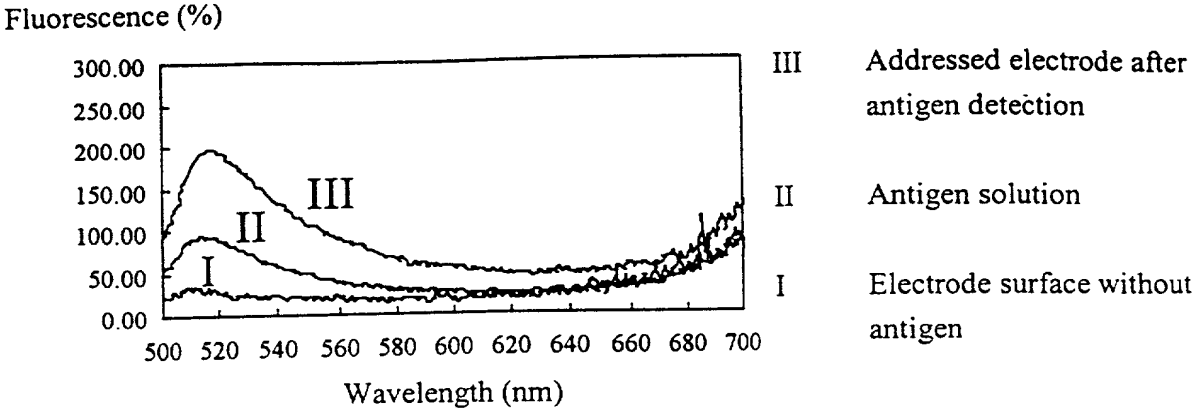

Fluorescence (%)
300.00
250.00
200.00
150.00
100.00
50.00
0.00
500 520 540 560 580 600 620 640 660 680 700
Wavelength (nm)
III
II
I
III Addressed electrode after antigen detection
II Antigen solution
I Electrode surface without antigen

Fig. 10

their duly appointed associates, my attorneys or agents, with full power of substitution and revocation, to prosecute this application, to make alterations and amendments therein, to file continuation and divisional applications thereof, to receive the Patent, and to transact all business in the Patent and Trademark Office and in the Courts in connection therewith, and to insert the Serial Number of the application in the space provided above, and specify that all communications about the application are to be directed to the following correspondence address:

William F. Lawrence, Esq.
c/o FROMMER LAWRENCE & HAUG, LLP
745 Fifth Avenue
New York, NY 10151
FAX (212) 588-0500

Direct all telephone calls to: (212) 588-0800
to the attention of:
William F. Lawrence

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

INVENTOR(S):

Signature: _____ Date: 05 05 00
Full name of sole or first joint inventor: Christian Miculka
Residence: ~~Gebeschusstrasse 36, 65929 Frankfurt, Germany~~ Dollingergasse 3/15, 1190 Wien, Austria
Citizenship: Austrian national

Signature: _____ Date: 17.4.00
Full name of second joint inventor: Norbert Windhab
Residence: Akazienstrasse 28, 65795 Hattersheim, Germany
Citizenship: German
Dr. Windhab

Signature: _____ Date: 22.5.2000
Full name of third joint inventor: Hans-Ulrich Hoppe
Residence: ~~Amselweg 11, 65929 Frankfurt, Germany~~ Meisenstraße 14, 85416 Langenbach, Germany
Citizenship: German

Post Office Address(es) of inventors [if different from residence]:

NOTE: In order to qualify for reduced fees available to Small Entities, each inventor and any other individual or entity having rights to the invention must also sign an appropriate separate "Verified Statement (Declaration) Claiming [or Supporting a Claim by Another for] Small Entity Status" form [e.g. for Independent Inventor, Small Business Concern, Nonprofit Organization, Individual Non-Inventor].

PATENT
514485-3810

DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY
(Includes reference to PCT International Applications)

FROMMER LAWRENCE & HAUG, LLP
File No.: 514485-3810

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and joint inventor (if plural, names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention ENTITLED:

ADDRESSABLE MODULAR RECOGNITION SYSTEM, PRODUCTION MODE AND USE

the specification of which:

is attached hereto
X was filed on March 20, 2000 as:
X United States Application Serial No. 09\509,051
X PCT Application No. EP98/06001 filed September 21, 1998
X with amendments through DATE EVEN HEREWITH (if applicable, give details).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code § 119 (a) - (d) or § 365 (b) of any foreign application(s) for patent or inventor's certificate, or § 365 (a) of any PCT International application(s) designating at least one country other than the United State of America listed below and have also identified below any foreign application for patent or inventor's certificate or any PCT International applications designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) on which priority is claimed:

Prior Foreign/PCT Application(s) [list additional applications on separate page]:

Country (or PCT)	Application Number:	Filed (Day/Month/Year)	Priority Claimed: Yes	No
Germany	197 41 716.7	22/09/97	X	

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below.

I hereby claim the benefit under Title 35, United States Code § 120 of any United States application(s) or § 365 (c) of any PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior United States or PCT International application(s) in the manner provided by the first paragraph of Title 35, United States Code § 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Prior U.S. (or U.S.-designating PCT) Application(s) [list additional applications on separate page]:

U.S. Serial No.:	Filed (Day/Month/Year)	PCT Application No.	Status (patented, pending, abandoned)
PCT	21/09/98	EP98/06001	pending

I hereby appoint William F. Lawrence, Registration No. 28,029, and FROMMER LAWRENCE & HAUG, LLP or